

Bulletin of the Agricultural Chemical Society of Japan.

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The Agricultural Chemical Society of Japan.

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The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

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On the Nutritive Value of Pentosan. I.

The Effect of Xylan on the Fat Formation.

By

Hisayoshi IWATA

(Merioka Imperial College of Agriculture and Forestry)

(Received May 5, 1930)

Introduction.

During his notable researches on the productive value of crude fiber and other nutrients, Kellner once conjectured that the fat forming value of straw pentosan would be equivalent to that of starch. No direct evidence or strict confirmation of this assumption has, however, ever been presented inspite of the abundant distribution of pentosans in feeding stuffs and food materials, especially in cereal straws.

So, taking xylan as one of the examples of pentosans, I have investigated its productive value in comparison with that of starch.

Experimental.

Xylan was prepared mainly from the alkaline extract of rice straw by the addition of alcohol. This xylan contained 85.7 % of organic matter, 86.2 % of which was xylan according to the yield of furfural phloroglucid or of non-fermentable sugar. Its total fuel value was 4167 calories per gram of organic matter.

Starch that was used as a comparison contained 80.6 % of organic matter with fuel value of 4136 calories per gram.

Metabolism experiments were carried on for about 100 days, dividing them into 3 or 4 periods of a definite diet such as basal ration, basal+xylan or basal+starch. As the basal ration 160 grams of the mixture, which consisted of 50% of "Genge" hay, 45% of barley powder and 5% of dried radish leaf powder, were used. The metabolism experiments on carbon and nitrogen, made afterwards, proved that this basal ration was adequate and slightly in excess of the maintenance requirement for both of the experimented animals. In the period of "basal+xylan" or "basal+starch" 20 grams of the air dried sample were daily added to the basal ration. On account of the occurrence of some amounts of digestibility depression, the digested amount of xylan was directly calculated from the digestibility coefficient of pentosan and the starch from that of the total inverted sugar.

The income and output of carbon and nitrogen were estimated from the analysed results of fodder, feces, urine and expired gas and also the nutritive ratio of each diet was calculated. Respiration experiments were made twice

a period applying Pettenkofer's respiration apparatus installed in professor Katayama's laboratory at the Kyushu Imperial University. It must be added here that the formation of methan gas by the rabbits were negligible.

The results of the metabolism experiments may be summarised in the accompanying table (Unit in grams):—

Rabbit		Rabbit No. I.							
Period		Basal		Basal + Starch		Basal + Xylan		Basal	
Carbon or Nitrogen		C	N	C	N	C	N	C	N
Income	Basal ration	64.51	3.176	64.51	3.176	64.51	3.176	64.51	3.176
	Sample	0	0	7.16	0.004	7.85	0.019	0	0
Out put	Feces	29.22	1.378	31.77	1.554	33.50	1.494	28.89	1.332
	Urine	2.26	1.600	2.20	1.381	2.13	1.391	2.28	1.683
	Expiration	31.80	0	34.76	0	33.86	0	32.63	0
Gain	Stored in body	1.23	0.198	2.94	0.245	2.87	0.310	0.71	0.161
Protein, stored		1.172		1.450		1.834		0.953	
Fat, stored		0.80		2.84		2.48		0.27	
Stored, sum as fat		1.49		3.70		3.56		0.83	
Nutritive ratio		6.04		7.79		7.21		5.90	

Rabbit		Rabbit No. II.					
Period		Basal + Starch		Basal		Basal + Xylan	
Carbon or Nitrogen		C	N	C	N	C	N
Income	Basal ration	64.51	3.176	64.51	3.176	64.51	3.176
	Sample	7.16	0.004	0	0	7.85	0.019
Out put	Feces	34.01	1.674	31.24	1.608	34.57	1.638
	Urine	2.10	1.298	1.81	1.347	2.13	1.291
	Expiration	31.62	0	31.10	0	31.40	0
Gain	Stored in body	3.94	0.208	0.36	0.221	4.26	0.266
Protein, stored		1.231		1.308		1.574	
Fat, stored		4.30		-0.43		4.48	
Stored, sum as fat		5.03		0.34		5.41	
Nutritive ratio		7.99		6.51		7.66	

The amount of fat accumulated in the body from one kilogram of digestible xylan or starch and also the percentage of production calorific value per calorific value of the digested nutrients were computed:—

Nutrients	Rabbit No.	Fat accumulated from 1 kg. digested nutrients	Production calorie
Xylan	1	197.2 g.	61.3%
	2	341.0 } average 269.1 g.	
Starch *	1	202.7	60.5%
	2	325.0 } average 263.9 g.	

The excreted urine of each period was analysed as to its total sugar as well as pentose contents; the amount of the latter was corrected for the presence of glucuronates and other urine constituents according to my equation,
 (Urine, Phloroglucid-glucuronate, Phloroglucid \times 0.422)

$$\div 0.692 \times 1.0784 = \text{Urine pentose.}$$

The results obtained may be tabulated as follows:—

Period	Rabbit No. 1				Rabbit No. 2		
	Basal	Basal + Xylan	Basal + Starch	Basal	Basal + Starch	Basal	Basal + Xylan
Total sugar per day mg.	455	481	510	446	446	403	500
Pentose " "	73	73	109	66	78	83	81

Conclusion

Nearly all of the digested xylan was metabolised by the rabbits so that mere trace of organic matter such as pentose etc. was excreted in the urine.

The productive value of digested xylan was equal to that of starch as Kellner had assumed. In other words, the amount of fat formation, calculated from the metabolism experiments on carbon and nitrogen, was 269 grams from one kilogram of digestible xylan and the productive calorific value, which was calculated from the accumulated fat, was 3029 calories per gram of digestible xylan, or the value corresponding to 61% of the calorific value of digested xylan.

On the Nutritive Value of Pentosan. II.

The Effect of Xylan Administration on the Glycogen Formation and Blood Composition.

By

Hisayoshi IWATA.

(Received May 7, 1931)

In the first report of this investigation it was concluded that, the amount of fat formed from the digested xylan is equal to that formed from starch.

According to this conclusion the amount of glycogen formed from xylan must also be equal to that formed from starch, but this point has not been experimentally investigated. Slowtsoff has once reported that, some amounts of pentosan could be found in the rabbit's body after the administration of xylan. The purpose of the present investigation was to ascertain the formation as well as the amount of glycogen or pentosan in the rabbits, administered xylan. The effect of xylan on the blood composition was also studied in this connection.

Methods

Male rabbits of medium weight were kept fasting in metabolic cages. Each animal was armed with a tin belly-band in order to prevent its access to feces. Room temperature was kept between 10° and 15° C. After a period of fasting ranging from 32 to 40 hours, either xylan or starch was administered into the stomach by the urethral catheter, in amount of 5 g. per 1 kg. of body weight. The blood samples were obtained from marginal ear veins at intervals using potassium oxalate as an anticoagulant. The blood sugar was analysed by the method of Hagedorn and Jensen, the reducing power after fermentation by an ordinary method, haemoglobin by the Sahli's haemometer method, and non-protein nitrogen by the Bang's micro Kjeldahl method. About 50 hours after fasting started, each rabbit was killed and its glycogen contents of liver as well as muscle were determined by the Pflüger's method. Glucose resulting from the hydrolysis of the glycogen was determined by the Bertrand's method. In order to ascertain the presence or absence of pentosan in the liver, the pentose content in the hydrolysed solution of glycogen, which was precipitated by the Pflüger's method as above noted, was determined both by the Tollen's method and by the fermentation method. The amounts of xylan, starch or sugar contained in the digestive tracts were determined by the usual method. The urine sugar was determined from the urine excreted as well as that remained in the bladder. The control rabbits were kept fasting 48 hours and passed through same examination as above mentioned.

Results

The results obtained may be shown by the following tables.

The amounts of glycogen stored in each rabbit were calculated as follows:

$$\text{(\text{Liver weight} \times \text{Liver glycogen } \%) + (\text{Body weight} \times 0.47 \times \text{Muscle glycogen } \%)}$$

The amounts of glycogen formed either from xylan or starch were calculated by the subtraction of the amounts of glycogen found in the control rabbits (fasted) from that of the animal administered. The results may be represented by the following graph.

Table I. Effect of Xylan.

Rabbit No.	Date.	Time (af. = after) (° = hrs)	Treatment and remark	Blood sugar mg. in 100c.c.	Nonferment- able blood sugar mg. in 100c.c.	Nonprotein nitrogen mg. in 100c.c.	Haemo- globin g. in 100c.c.	Liver weight g.	Liver glycogen mg. in 100 g.	Muscle glycogen mg. in 100 g.
1	Oct. 21 23	am. 10.0	Fasting started.							
		" af. moment af. 4° af. "	Body weight 1.8 kg. 10 g. Xylan given. Blood taken. " " Killed 52° after fasting Body weight 1.68kg.	105 93	39 37	48 52	17.0 17.1	56	78	39
2	Nov. 11 12 13	am. 10.30 " 11.30 p.m. 4.30 af. 17°10' af. 18° 0	Fasting started. Blood taken. Body weight 1.9 kg. 10 g. Xylan given. Blood taken. Killed 48° after fasting. Body weight 1.75kg. Urine sugar 110 mg. for 18°. 1 g. Xylan recovered in stomach.	108 115	43 46	51 46	16.4 17.0	59	1139	174
3	Dec. 25 27	p.m. 3.00 a.m. 7.55 af. 25' af. 4° af. 8°05 af. 9°15	Fasting started. Body weight 1.44 kg. 8 g. Xylan given. Blood taken. " " Killed 50°10' after fasting. Body weight 1.3 kg. Urine sugar 173 mg. for 9°. 1 g. Xylan recovered in stomach.	92 — 85	41 43 38	49 44 54	15.9 16.1 16.0	40	890	97
4	Dec. 26 27 28	a.m. 8.35 a.m. 9.25 p.m. 5.20 p.m. 5.30 af. 15°50/ af. 16°20'	Fasting started. Blood taken. " " Body weight 1.84 kg. 8 g. Xylan given. Blood taken. Killed 49°15' after fasting. Body weight 1.75 kg. Urine sugar 235 mg. for 16°. 1.5 g. Xylan recovered in stomach.	102 108 117	32 36 35	53 — 56	15.9 — 15.9	57	749	161

5	Jan. 9 10	a.m. 9.10 p.m. 5.30 p.m. 5.45 af. 15°15' af. 15°30'	Fasting started. Blood taken. Body weight 1.8 kg. 9 g. Xylan given. Blood taken. Killed 48°05' after fasting. Body weight 1.65 kg. 0.7 g. Xylan recovered in stomach, and intestine.	99 112	31 32	— 47	17.1 16.9	52	1493	90
6	Feb. 5 7	p.m. 1.40 a.m. 8.40 af. 5' af. 7°50' af. 8°	Fasting started. Body weight 1.9 kg. 10 g. Xylan given. Blood taken. " " Killed 51° after fasting. Body weight 1.8 kg. Urine sugar 86 mg. for 8°. 1.85 g. Xylan recovered in caecum, 0.3 g. in stomach.	87 98	29 31	46 43	17.3 17.1	62	1020	220
			Average (No. 2~6)						1058	149

Table II. Effect of Starch.

Rabbit No.	Date.	Time (af. = after) (° = hrs)	Treatment and remark	Blood sugar mg. in 100c.c.	Nonferment- able blood sugar mg. in 100c.c.	Nonprotein nitrogen mg. in 100c.c.	Haemo- globin g. in 100c.c.	Liver weight g.	Liver glycogen mg. in 100 g.	Muscle glycogen mg. in 100 g.
7	Oct. 3 5	p.m. 0.10 p.m. 0.30 af. moment " 2°50' " 4°	Fasting started. Body weight 1.95 kg. 10 g. Starch given. Blood taken. " " Killed 52°20' after fasting. Body weight 1.82 kg.	92 98	31 36	44 49	17.3 17.3	60	405	92
8	Oct. 9 11	P.m. 0 " " af. moment " 2°20' " 3°50' " 4	Fasting started. Body weight 1.70 kg. 10 g. Starch given. Blood taken. " " Killed 52° after fasting. Body weight 1.55 kg.	92 93 103	38 — 40	50 — 49	17.3 17.3 17.0	46	274	75

9	Oct. 29 Nov. 1	p.m. 0.30 " 25/ af. 2° " 4°05/ " 4°20/	Fasting started. Body weight 1.5 K. 10 g. Starch given. Blood taken. " " " " Killed 52°20' after fasting. Body weight 1.5 K.	79 93 87	— — —	— — —	15.4 15.2 15.6	50	326	84
10	Nov. 18 19 20 20~26 Nov. 26 27 28	a.m. 10.30 " 10.30 " 10.35 p.m. 4.30 a.m. 10.30 a.m. 9.20 p.m. 4.0 af. 17°20/	Fasting started. Blood taken. 7g. Starch given. Blood taken " " Normal feeding. Fasting started again. Body weight 1.35 kg. 6.5 g. Starch given. Killed 48° after fasting. Body weight 1.32 kg.	96 94 103	42 — 40	41 39 44	17.1 17.0 17.3	39	727	62
11	Dec. 9 10 11	a.m. 9.30 " 10.00 p.m. 4.00 " 4.30 af. 17°35/ " 18°	Fasting started Blood taken. " " Body weight 1.65 kg. 8 g. Starch given. Blood taken. Killed 49° after fasting. Body weight 1.64 kg. Urine sugar 213 mg. for 18°. 2 g. Starch recovered in stomach.	108 90 125	41 — 45	39 37 39	17.0 16.4 17.0	50	660	138
12	Dec. 25 27	p.m. 4.00 a.m. 8.40 af. 20/ " 3°55/ " 8°20/ " 9°	Fasting started. Body weight 1.85 kg. 8 g. Starch given. Blood taken " " " " Killed 49°40' after fasting. Body weight 1.38 kg. Urine sugar 189 mg. 1.4 g. Starch recovered in stomach.	93 88 —	41 — 40	50 — 52	15.6 16.0 15.9	42	704	87
13	Jan. 6 8	p.m. 5.00 a.m. 9.00 " 9.15 af. 7°15/ " 8°	Fasting started. Blood taken. Body weight 1.8 kg. 9 g. Starch given. Blood taken. Killed 48°15' after fasting. Body weight 1.81 kg. 0.7 g. Starch recovered in stomach.	104 105	46 40	48 45	16.2 16.5	62	1538	356
			Average (No. 10~13)						907	161

Table III. Effect of Fasting.

Rabbit No.	Date. 1930 1931	Time (° = hrs)	Treatment and remark	Blood sugar mg. in 100c. c.	Nonferment- able blood sugar mg. in 100c. c.	Nonprotein nitrogen mg. in 100c. c.	Haemo- globin g. in 100c. c.	Liver weight g.	Liver glycogen mg. in 100 g.	Muscle glycogen mg. in 100 g.
14	Oct. 5 6 7	a.m. 10.0 p.m. 2.0 a.m. 10.0	Fasting started. Blood taken. Body weight 1.84 kg. Killed 48° after fasting. Body weight 1.8kg.	108	39	46	17.1	50	109	90
15	Oct. 12 13 14	a.m. 10.00 " 9.30 " 9.30 " 10.00	Fasting started. Blood taken. Body weight 1.70 kg. " " Killed 48° after fasting. Body weight 1.6kg.	86 78	43 40	48 52	16.9 17.5	41	74	32
16	Nov. 8 10	p.m. 3.0 " 3.0	Fasting started. Killed 48° after fasting. Body weight 1.3kg.					44	73	41
17	Jan. 10 11 12	a.m. 10.00 p.m. 5.00 a.m. 9.50 " 10.00	Fasting started. Blood taken. Body weight 1.5 kg. " " Killed 48° after fasting. Body weight 1.45kg. Urine sugar 243 mg. for 18°	93 89	38 35	48 47	16.7 16.9	45	133	39
18	Jan. 10 11 12	a.m. 10.00 p.m. 5.10 a.m. 10.30 " 10.35	Fasting started. Blood taken. Body weight 1.4 kg. Blood taken. Killed 48° ³⁵ after fasting. Body weight 1.38 kg. Urine sugar 195 mg. for 20°.	87 86	41 44	44 43	— 16.9	45	108	79
			Average (No. 14~18)						99	56

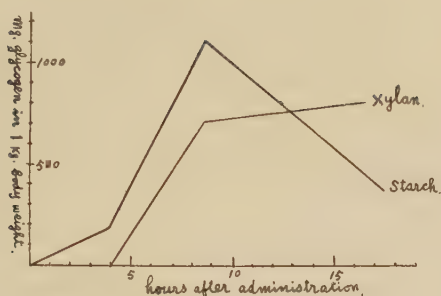


Fig. Glycogen increased in Liver and Muscle.

The presence or absence of pentosan in the liver of rabbits, to which xylan was administered, was investigated with the following results.

Table IV. Pentosan (?) found in Liver.

Administered	Rabbit No.	Time	Pentosan (?) %	
			By Tollen's method.	By fermentation method.
Xylan	4	1930, Dec. 26~28	0.019	—
	5	1931, Jan. 9~11	0.036	0.04
	6	" Feb. 5~7	0.011	—
Starch	10	1930, Nov 26~28	0.011	—
	12	" Dec. 25~27	0.021	—
	13	1931, Jan. 6~8	0.088	0.05

Conclusion

1. The amount of fermentable blood sugar was found increased by 0.1 %, 8~10 hours after the administration of xylan, but the amounts of non-fermentable blood sugar, non-protein nitrogen and haemoglobin remained unchanged.

2. No pentosan was found in the rabbit's liver even when some amounts of xylan was administered.

3. The total amounts of glycogen formed by the administration of xylan were nearly equal to that formed when the same amount of starch was given.

4. The results above stated is a strict confirmation of the conclusion, stated in the first report i.e. the nutritive value (dynamic energy) of the digested xylan is equal to that of starch so far as the rabbit, nay, probably the herbivorous animals are concerned.

5. Digestion velocity of xylan is smaller than that of starch.

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2. Slowtsoff: Hoppe-Segler's Zeitsch. f. physiol. Chemie, **34**, 181, (1901).

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Investigation on Cellulose Decomposition in Soils. I.

Re-examination of some stock cultures.

By

Arao ITANO and Satiyo ARAKAWA.

(Received May 2, 1931)

An enquiry was made as to the causal agent of forming the clear zone around the colony when the cellulose decomposing bacteria are cultivated on cellulose agar since there has been a dispute among various investigators, if the zone is formed by the enzymatic action or acid which is produced as the metabolic product.

The following cultures which were obtained from American Type Culture Collection, were used: *Cellulomonas flavigena*, *fibula*, *fima*, *liquata*, *biazotea*, *cellasea*, *gelida*, *perurida* and *uda*.

Various media were used such as McBeth, Löhnis and Lochhead, Skinner, Bradley and Rettger, Snieszko, Dubos and the author's media, a) feces and b) yeast extract.

The following summary are given:

1) Genus, *Cellulomonas* produce the enzymatic zones on cellulose agar medium; 2) the kind of media plays an important rôle and the organic nitrogen is the best source of nitrogen especially the digested casein and yeast extract; 3) all the cultures retained their activity after years storage although *Cellulomonas uda*, *fima* and *gelida* manifested strong power of the cellulose decomposition while *Cellulomonas cellasea* was the weakest.

Investigation on Electrometric Method for Determination of Chloride.

By

Arao ITANO.

(Received May 2, 1931)

The use and setting up of portable pH apparatus (Itano) is described in conjunction with the Best's electrometric method for determination of chloride which uses a reference electrode of known potential.

The reference electrode is made up of a buffer solution exclusive of chloride, pH 3.00~3.03 to which quinhydrone is added. The silver metal

electrode coated with silver chloride by electrolysis is immersed in a sample and used as an indicator electrode. These two electrodes are connected to form a chain by means of an agar bridge which is made with saturated KCl and 3% agar. The chain is connected to the apparatus and the titration is carried out with the standard silver nitrate added from a burette. The end-point of titration is indicated by the reverse direction of galvanometer needle.

The special merits of the method are summarized as follows: 1) the end-point is sharp and easily observed which avoids the personal factor; 2) no necessity of making graph or any other interpolation like in case of other electrometric method; 3) each determination is carried out quickly, and no color and turbidity interferes with the method.

The portable pH apparatus (Itano) is very conveniently employed in completing the necessary equipment.

Determination of Chloride in Soils.

By

ARAO ITANO and AKIRA MATSUURA.

(Received May 2, 1931)

The electrometric method described by the senior author is used in determining the chloride content in the soil samples which were collected along the irrigation canal running from the Takahashi river to the Kojima Bay in Okayama prefecture. Some samples were taken from the arable rice-field and the others; from the virgin field located on the bank or basin of the canal. The chromate method was applied at the same time to check each other.

The following summary and conclusions are given: 1) the results obtained by the electrometric method are in close agreement with those obtained by the standard chromate method; 2) there is a slight discrepancy among the results when the chloride content is very low; 3) the chloride content decreases gradually toward the source of irrigation in both arable and virgin soils; 4) the virgin soils contain much more chloride naturally than the arable soils of which the chloride has been gradually washed out; 5) the organic matter contained as much as in these samples does not interfere with the determination.

Chemical Studies of Agar-Agar.

I. The Rate of Hot-Water-Hydrolysis of Agar-agar.

By

Eiji TAKAHASHI and Kiyoshi SHIRAHAMA.

(Received May 8, 1931)

We are now studying the various carbohydrates which separated from the "hydrato-kanten*" obtained by hot-water-hydrolysis of agar-agar. In this paper the rate of hydrolysis of various agar-agars and the general nature of the hydrolyzed solution are reported.

Samples used in these experiments were a purified bar kanten made in Japan (made mainly from *Gerdium Amansii*, Lamx.), Merk's agar-agar and that made in Karafuto (made from *Ahnfeltia plicata*).

The agar-agar and water were mixed in the ratio of 1 to 20 in weight and heated in autoclave at 130°C for certain hours of various intervals. Chemical and physico-chemical investigations were made upon the above resultant solutions and also the specific viscosity (I), hydrogen ion concentration (II), reduction value (III), aldose value (IV), free-sulfuric acid (V) and the quantity of humus (VI) were measured as follows:—

Table I.

The rate of hot-water-hydrolysis of Japanese bar Kanten

Time of heating (Hours)	I	II (PH)	For 100 g. of Kanten			
			III*	IV	V	VI
2	2.514	4.64	5 g.	12 g.	0.10 g.	—
3	1.118	3.49	23	30	0.15	—
4	1.109	3.29	23	31	0.26	1.48 g.
5	1.109	3.08	28	33	0.75	1.50
6	1.030	2.80	50	39	1.53	2.24
7	1.020	2.52	55	42	2.25	3.60
8	1.009	2.04	55	43	3.11	4.64

* Calculated as a galactose.

Table II.

The rate of hot-water-hydrolysis of various agar-agar.

Time of heating (Hours)	Specific viscosity			Per cent for agar-agar					
				Reducing sugar			Free-sulfuric acid		
	B	M	K	B	M	K	B	M	K
2	2.51	—	—	5	—	—	0.10	—	—

* All hydrolyzed products of agar-agar.

3	1.12	1.83	4.04	23	12	5	0.15	0.22	0.03
4	1.11	1.25	1.70	23	19	13	0.26	0.37	0.07
5	1.11	1.20	1.40	28	30	28	0.75	0.59	0.08
6	1.03	1.15	1.18	50	44	28	1.53	0.67	0.13
7	1.02	1.07	1.12	55	51	29	2.25	0.85	0.18
8	1.01	1.00	1.09	55	51	42	3.11	0.96	0.24

B.....Bar kanten

M.....Merk's agar-agar

K.....Karafuto kanten

When the above result is observed, tracing curves (ordinate.....factors, abscissa.....time of heating), it is found that the two minimum points of the viscosity coincide respectively with the two maximum points of the curves of the reduction value.

The marked descending of viscosity at the early stage may depend upon the disappearing of starchy substance which appears in the hydrolyzed solution heated for 2 hours in the case of the bar kanten. In the course of cooling of the resultant hydrolyzed solution, it exists in a white turbid state at first, but is precipitated soon after forming a starchy-like layer, the upper solution remaining clear.

It was also indicated that the aldose value is higher than the reduction value.

Protein of "Wakame" (*Undaria pinnatifida*).

I. Hydrolysis of "Wakame".

By

Shun'ichi TASE.

(Received May 12, 1931)

The material of Wakame was hydrolysed with hydrochloric acid. The proportion of various forms of amino nitrogen in the hydrolysed solution was determined by the Van Slyke's method as follows:—

The proportion of Amino Nitrogen in Wakame.

	Percentage in total N.	Percentage in the total soluble N, after hydrolysis
Soluble N.	92.01	
Insoluble N. (in the residue after hydrolysis)	8.75	
Ammonia N.	7.87	8.55
Melanin N.	11.63	12.64
Precipitated with $\text{Ca}(\text{OH})_2$.	3.51	3.81
Not precipitated with $\text{Ca}(\text{OH})_2$, but with phospho- tungstic acid.	8.12	8.83

Basic N.	6.59	7.16
Free amino N.	3.92	4.27
Non-amino N.	2.67	2.89
Arginine N.	3.29	3.58
Cystine N.	2.93	3.18
<hr/>		
Nitrogen in the filtrate from base.	61.69	67.04
Free amino N.	54.55	59.29
Non-amino N.	7.14	7.75

From another one kilogram of the same sample various pure amino acid crystals were separated by the ester method. Yields were as follows:

Yields of Amino Acids from 1 kg. of Wakame:

Amino Acid	Yields (g.)	Amino Acid	Yields (g.)
Glycocoll	0.80	Glutamic acid	8.90
Alanine	8.15	Proline	2.90
Valine	0.78	Anhydride of amino acid	0.08
Leucine	1.15	Phenylalanine hydrochloride (?)	5.36
Aspartic acid	1.58		

Studies on the Dietary Properties of Soybean Cake Flour Produced by the Alcohol Extraction Process.

I. Digestibility of Protein Supplied by Alcohol Extracted Soybean Cake.

By

Seiichi IZUME and Yoshinori YOSHIMARU.

(From the Central Laboratory, South Manchuria Railway Co.)

(Received May 11, 1931)

Introduction

Although soybean has been used to a considerable extent for food purposes in Japan and China, the soybean cakes, by-products of oil mills, have not yet been successfully introduced into the human dietary, a tremendous amount of the cakes produced in Manchuria being at present utilized almost exclusively as fertilizers and stock feed.

Some of the chief reasons why soybean cakes find such a limited use as human food may be pointed out as follows:

(1) The soybean cakes commonly produced in Manchurian oil mills, both press-cake and benzine extracted cake, possess a somewhat disagreeable odor and unpleasant taste and are of a yellow or brown color.

(2) They contain sand, dust, grasses and various other impurities as the oil is not expressed or extracted under sanitary conditions.

(3) The round press-cake easily becomes mouldy and rancid as a result of its high moisture content.

Dr. Sato and his co-workers⁽¹⁾ of our laboratory recently invented a new process of extracting the oil from soybean by the use of alcohol as a solvent and were able to produce, on a semi-industrial scale, soybean cake with a finer appearance than any of the cakes commonly produced, it being devoid of the distasteful qualities above-mentioned. Such physical qualities as well as the high protein content of the product suggest the greatest possibilities of its providing in the near future a new important source of protein in the human dietary.

In this series of investigations we wished to study the nutritive value of soybean alcohol extracted cake as well as the value of its protein to supplement those of common cereals. According to Ishida⁽²⁾ the soybean cake produced by the alcohol extraction method contains a much larger amount of denatured protein than those obtained by extraction with other solvents like benzine and benzene, and he accordingly suggested that the protein of alcohol extracted cake may not possibly be well utilized by the animal organism. We thought, therefore, that it would be of the utmost importance, in recommending this new kind of soybean cake for human food, to estimate to what extent its protein can be utilized by human beings.

In the first paper we reported on the results of our digestive experiments in which the coefficients of the digestibility of protein supplied by alcohol extracted cake were determined on both white rats and humans.

Experimental Results

Test Materials.

The samples of alcohol extracted and benzine extracted soybean cake flours were supplied by the Experimental Soybean Oil Plant in our laboratory and that of round press-cake was obtained from the Nisshin Oil Mill of Dairen.

The alcohol extracted cake flour used in the experiments was prepared as follows: 40 kg. of soybean were crushed by iron rollers, dried with hot air and heated at about 80°C for 3 hours with 140 L. of ethyl alcohol (95~6 per cent) in a closed rotary extractor. During the extraction a portion of hot alcoholic solution was continuously removed from the extractor and passed through a cooler and after using the separator to take off the dissolved oil it was recirculated into the extractor. At the end of the operation the alcoholic solution was drawn off and the residue remained in the extractor was dried under a reduced pressure with a stream of hot air at 60~70°C. The

cake thus obtained was then subjected to a milling machine by which it was ground into a flour and passed through 124 mesh sieves, the majority of skins of soybean being removed by sifting.

The alcohol extracted soybean cake flour as well as other test materials employed in the experiments had the chemical composition as shown in Table I.

Table I.

Kind of Soybean Flour	Moisture %	Ash %	Crude Fat %	Crude Protein %	Nitrogen		H ₂ O-Sol. N. in Total N. %
					Total %	H ₂ O- Soluble %	
Unextracted Soybean	8.4	5.7	17.5	42.5	6.8	6.0	88.3
Alcohol Extracted Cake	8.1	6.2	1.4	56.9	9.1	2.2	24.2
Benzine Extracted Cake	7.6	6.3	1.3	56.2	9.0	7.4	82.2
Round Press-Cake	10.5	5.9	7.4	46.9	7.5	3.4	45.3

Digestive Experiments on Rats.

To each group consisting of 6~8 rats weighing about 100 g. were given 12 experimental diets which contained the proteins supplied by soybean, its alcohol extracted, benzine extracted and pressed cakes at 10, 15 and 20 per cent, together with starch McCollum's salt mixture No. 185, soybean oil, cod liver oil and oryzanin, this being a vitamin B preparation. In preparing the diets these ingredients were thoroughly mixed with water and cooked for about 2 hours in a gas oven.

The experimental period lasted for one week, during which the total nitrogen of rations taken by each group of rats as well as that of feces excreted by them were noted daily to estimate the coefficients of apparent digestibility at different intake levels for the proteins supplied by these test materials. For the calculation of true digestibility some corrections based on the excretion of nitrogen derived from metabolic products and from intestinal bacteria were made, rats weighing about 100 g. being found to have excreted on an average 0.075 g. of nitrogen per week when fed with a nitrogen-free ration.

As indicated in Table II it was shown that the coefficients of apparent digestibility of proteins were much influenced with the amount of proteins ingested by rats while those of true digestibility were almost independent from the protein intake, their average values being estimated as follows: soybean flour 84.8 per cent, alcohol extracted cake flour 85.0 per cent, benzine extracted cake flour 85.7 per cent and press-cake flour 84.4 per cent.

From the comparison of these utilization figures it is evident that the protein of alcohol extracted cake, inspite of its marked denaturation, was digested by rats almost in an equal degree with those supplied by soybean and other kinds of its cakes containing less amounts of denatured proteins.

Table II.

Experimental Diet		Amount of N in Diets consumed	Amount of N in Feces	Coef. of Digestibility	
Test Material	Protein Content			Apparent	True
Unextracted Soybean Flour	10%	5.51 g.	1.267 g.	77.0%	85.2%
	15	6.28	1.483	76.4	83.6
	20	8.87	1.732	80.5	85.6
				Average	84.80
Alcohol Extracted Cake Flour	10	7.93	1.692	78.7	84.4
	15	10.51	2.281	78.3	84.0
	20	12.97	2.359	81.8	86.5
				Average	84.97
Benzine Extracted Cake Flour	10	4.58	1.049	77.1	86.9
	15	7.27	1.552	78.7	84.9
	20	12.86	2.498	80.6	85.2
				Average	85.73
Round Press-Cake Flour	10	7.56	1.884	75.1	83.0
	15	8.07	1.598	80.2	85.8
	20	16.07	3.129	80.6	84.3
				Average	84.37

Digestive Experiments on Humans.

Bread and biscuits prepared with the mixtures of wheat flour and alcohol extracted soybean cake flour (20~40 per cent) were given to 4 men for 6 days of experimental period which succeeded the preliminary period of 3 days during which the subjects ate plain bread and biscuits instead of soya-bread and soya-biscuits as principal foods. During the entire periods they were requested to take definite amounts of accessory foods every day as follows: breakfast, 1 apple; lunch, 90 g. "carnation milk" and 20 g. sugar; dinner, 2 fried eggs and 200 g. the leaf of "hakusai", *Brassica Campestris* L. Tea and water were to be taken in liberal quantities.

The amount of food proteins taken by individual subject during the experimental period varied from 543.8 to 912.5 g. 41~47 per cent of which were derived from the soybean cake; no one experiencing any physiological abnormalities.

To estimate the coefficients of digestibility for the protein of soybean cake it was assumed that the proteins supplied by wheat flour, egg, milk, hakusai and apple were 88, 97, 94, 85 and 85 per cent digested respectively.

The experimental results were shown in Table III in which we estimated that the protein supplied by soybean alcohol extraction cake was 84.7~91.8, or on an average 88.6 per cent utilized by these subjects. Holmes⁽³⁾ who fed 7 men with biscuits made with a mixture of wheat flour and soybean press-cake flour together with fruits, butter and sugar calculated that the

protein supplied by soybean press-cake was 79.4~90.2 per cent, or approximately 85.3 per cent digested by them.

Table III.

Subject, Name Age Body Weight	C.K.T. 21 54.0 kg.		C.K.M. 15 35.5 kg.		C.C.R. 18 57.5 kg.		S.I. 33 53.5 kg.	
Foods consumed	Weight of Food	N in Food	Weight of Food	N in Food	Weight of Food	N in Food	Weight of Food	N in Food
	g.	g.	g.	g.	g.	g.	g.	g.
Soya-Bread	4930	105.98	2802	60.24	4512	97.01	2481	53.34
Soya-Biscuits	796	18.23	718	16.44	1206	27.62	552	12.64
Apple	713	0.28	780	0.31	777	0.31	625	0.25
Egg	585	11.45	637	12.46	627	12.28	618	12.12
Butter	420	0.67	240	0.38	420	0.67	240	0.38
Carnation Milk	540	5.94	540	5.94	540	5.94	540	5.94
Sugar	120	—	120	—	120	—	120	—
Leaf of Hakusai	1200	2.40	600	1.20	1200	2.40	1200	2.40
Total	9304	144.95	6437	96.97	9402	146.23	6376	87.07
Calorie Intake per day (average)	3419 Cal.		2330 Cal.		3535 Cal.		2101 Cal.	
Feces excreted								
Total Solid Matter	187.0 g.		154.0 g.		263.0 g.		112.0 g.	
Total Nitrogen	14.00		10.63		18.39		7.70	
N derived from undigested soybean protein	5.94		5.52		10.51		2.97	
Coef. of Digestibility								
Total Food Protein	90.34%		89.00%		87.42%		91.15%	
Average	—		—		—		89.48	
Soybean Protein	91.07		86.96		84.71		91.77	
Average	—		—		—		88.62	

Based on the comparison between these utilization figures it may be justified to conclude that the protein of soybean alcohol extraction cake is comparatively well digestible by men, its coefficient of digestibility being just as large as that of protein supplied by soybean press-cake.

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II. On the Nutritive Value of Soybean Alcohol Extracted Cake.

By

Seiichi IZUME, Yoshinori YOSHIMARU and Isao KOMATSUBARA.

Introduction

The facts that soybean contains a large amount of physiologically good

protein and is rich in the water-soluble growth factor were first demonstrated by Daniels and Nichols⁽¹⁾, and Osborne and Mendel⁽²⁾ and were subsequently observed by a number of investigators.

It was also shown by Osborne and Mendel that the protein supplied by soybean press-cake was not inferior in the nutritive value to that of soybean. According to Ohtomo⁽³⁾ the protein supplied by soybean benzine extracted cake was noticeably inferior in the physiological value to that derived from press-cake. He also demonstrated that the nutritive value of soybean cakes was decreased to certain extents by treating them with hot alcohol; he ascribed these facts to the denaturation of soybean protein caused by the application of the solvent and heat. His conclusion were, however, not very convincing, being but poorly supported by the results of his feeding experiments.

In the present investigations we compared the nutritive value of the protein of alcohol extracted cake with those supplied by soybean, its benzine extracted and pressed cakes and tried to ascertain whether the denaturation of protein was the determining factor in the evaluation of nutritive efficiency of soybean protein. We also made comparisons between soybean and its alcohol extracted cake for the values in furnishing essential vitamins.

Experimental Results

The Nutritive Value of Protein Supplied by Soybean Alcohol Extracted Cake Flour.

In evaluating the nutritive value of protein supplied by soybean alcohol extracted cake young rats weighing about 40~50 g. were placed on experimental diets for periods varying from 140 to 240 days, and the rates of their growth, fertilities of female rats as well as the abilities of mothers to rear their young were carefully observed. The diets contained the alcohol extracted cake as the sole source of protein which supplied 7.5, 10, 15 and 20 per cent protein and were supplemented with other essential nutrients, such as, starch, McCollum's salt mixture (5%), cod liver oil (3%), soybean oil (7%) and oryzanin (5 c.c. per 100 g. of diet).

The rats, when fed upon a diet (No. ACMa) including the protein supplied by alcohol extracted cake* at 7.5 per cent, grew at a slow rate; their body weights seldom reaching 150 g. in 140 days of the experimental period, and of which none of the females successfully reproduced. With a diet (No. ACMb) containing protein at 10 per cent, rats acquired a better growth, but their growth curves were decidedly inferior to those of normal nutrition observed by Osborne and Mendel. No offsprings were observed to have been produced on this diet. When the protein content of diet was

increased to 15 per cent (Diet No. ACMc) the growth rates were much improved; the females often producing the young but failing to suckle them successfully in most cases, but when the protein formed 20 per cent of diet (No. ACMd) rats secured a normal growth and the females produced normal litters of young which were normally suckled. The growth curves shown by rats placed on these four kinds of experimental diets are indicated in Figure I.

We also conducted a number of feeding experiments under the otherwise similar conditions employing soybean flour, soybean benzine extracted and pressed cake flours as test materials. The diets contained the proteins supplied by these materials at 7.5, 10, 15 and 20 per cent, together with other nutrients similar to those employed in the previous experiments. The experimental results demonstrated that these proteins of different preparations with their various contents of denaturated proteins were very similar in their nutritive values to that of soybean alcohol extracted cake, when employed as the sole source of protein.

These facts prove that the physiological value of soybean protein is not significantly altered by heating it under the described conditions with such solvents as alcohol or petroleum benzine, although alcohol causes a marked physical change (denaturation) of protein. Our results also confirm those of previous investigators who have demonstrated that soybean contains protein of a high physiological value.

*Comparison of the Vitamin A Value between Soybean and its Alcohol
Extracted Cake.*

The fact that the addition to diets of soybean protein at 20 per cent supplied a sufficient amount of protein for a normal growth of rats induced us to use soybean and its alcohol extracted cake as a means of supplying both vitamin A and protein in the diets for the evaluation of the vitamin A factor of these test materials.

One diet consisted of 47 g. of soybean flour which supplied 20 per cent protein with 45 g. of starch, 5 g. of McCollum's salt mixture, 3 c.c. of irradiated ergosterol solution (0.001 per cent in olive oil) and 5 c.c. of oryzanin. Two other diets were composed of 35 and 53 g. of alcohol extracted cake flour which furnished 20 and 30 per cent protein, 50 and 32 g. of starch and 7 and 7 g. of soybean oil, besides the same amounts of inorganic salts, irradiated ergosterol and oryzanin as were contained in the former diet.

Young rats showed when placed on the diet which was 47 per cent soybean a fairly rapid gain of weight at an initial period of 3~5 weeks which was followed by a retardation and cessation of growth, after which the animals finally died as they developed ophthalmia due to the lack of vitamin

A. The addition to the diet of 3 per cent of cod liver oil, however, was followed by a prompt recovery from the eye disease and an immediate resumption of growth, the fact proving that soybean flour was not great in the vitamin A value.

On the diets in which soybean alcohol extracted cake flour was added at 35~53 per cent the majority of rats showed a less rapid growth at the beginning and developed ophthalmia, dying sooner than those on the previous diet.

These experimental results indicate that while the vitamin A values of both soybean and its alcohol extracted cake are not considerably great, the latter is undoubtedly inferior in the content of this factor.

Comparison of the Vitamin B Value.

Our procedure to evaluate the vitamin B factor of soybean and its alcohol extracted cake involved the feeding of young rats on diets in which the test materials were incorporated as the sole sources of both vitamin B complex and protein, and observing their power to promote the growth of animals. The experimental diets contained 47 per cent soybean or 35~53 per cent alcohol extracted cake and were equally supplemented with starch, McCollum's salt mixture (5%) and cod liver oil (3%), their protein contents varying from 20 to 30 per cent.

Two specimens of alcohol extracted cake were tested for the vitamin potency. One of them "No. A" was obtained from soybean by extraction with fresh alcohol and the other one "No. B" by treating it with the alcoholic solution which had been repeatedly used in the previous extraction processes for more than ten times.

The rats placed on the diets containing 47 per cent soybean as well as those on the ration 53 per cent of which was alcohol extracted cake "No. B" were capable of attaining normal growth, while those on the diets including alcohol extracted cake "No. A" at 35~53 per cent were unable to secure a good growth, their growth rates being much inferior to those of normal nutrition.

Conclusions may be drawn from these results that while soybean contains a fairly abundant quantity of vitamin B, the value of alcohol extracted cake for this factor is dependent on the kind of solvents used in the extraction; the cake obtained by means of fresh alcohol is not great in this essential while the one produced by extraction with the alcoholic solution which had been repeatedly used in the previous extraction is shown to be quite potent in this factor, its value being not much different from that of soybean.

Comparison of the Vitamin D Value.

Young rats were placed on the experimental diets for 4 weeks and at the end of the period the rachitic changes produced at the distal end of the tibia were radiographically examined. The diets consisted of 24~47 per cent soybean or of 18~53 per cent its alcohol extracted cake, besides starch, butter (5%), calcium carbonate (3%), sodium chloride (2%) and oryzanin. The control animals while placed on the same diets were daily fed with 1/100 mg. of irradiated ergosterol.

The results of experiments showed that while the control animals produced no rachitic lesions all the rats placed on the diets containing soybean at 24~48 per cent as well as those on the diets including 18~53 per cent alcohol extracted cake were demonstrated to have been afflicted with rickets, the fact indicating that both soybean and its alcohol extracted cake have comparatively little vitamin D value.

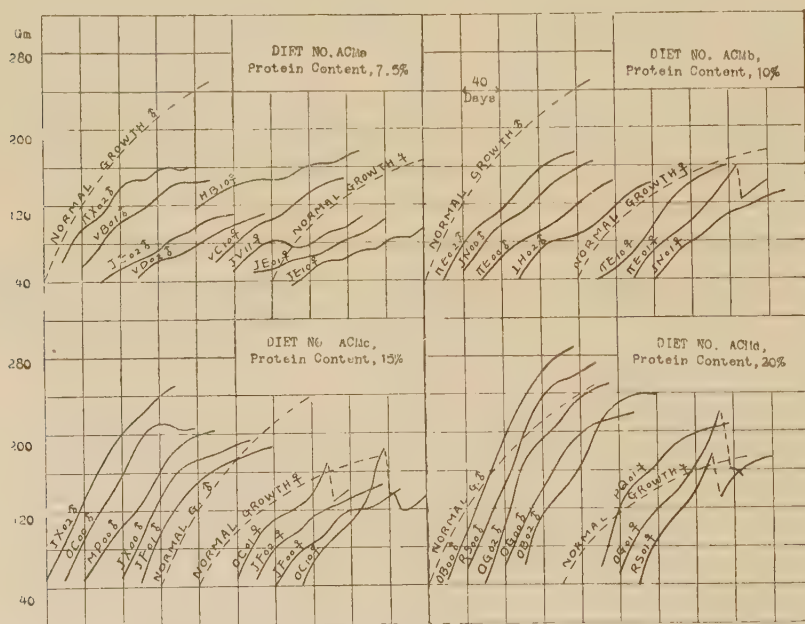


Fig. 1. The growth curves of rats placed on diets containing various amounts of protein supplied by alcohol extracted soybean cake as the sole source of protein.

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III. Supplementary Dietary Relations of the Protein of Soybean Cake for those of Cereals.

By

Seiichi IZUME and Isao KOMATSUBARA.

Introduction

Such cereals as wheat, rice, maize and kaoliang, commonly used as human foods, are relatively poor in the protein content and their proteins are more or less incomplete in satisfying the physiological requirements of the animal organism, being deficient in some essential amino-acids, especially in lysine. Soybean and its cake, on the other hand, contain a large amount of proteins which are comparatively rich in lysine. It is, therefore, naturally expected that the addition of soybean or its cake to the above cereals might elevate the nutritive values of cereals by increasing their protein contents and by improving the qualities of their proteins.

It was already demonstrated by Osborne and Mendel⁽¹⁾, McCollum, Simmonds and Parsons⁽²⁾ and Johns and Finks⁽³⁾ that the protein of soybean was capable of supplementing efficiently those of wheat and maize proteins. The present investigations were undertaken with the intention of demonstrating as to what extent the mixtures of soybean alcohol extracted cake flour with the flours of these cereals were superior in the nutritive value to those of cereal flours alone, provided adequate amounts of various vitamins and inorganic salts were similarly supplemented. The values of the protein supplied by alcohol extracted cake in supplementing the proteins of cereals were also studied.

Experimental Results

Test Materials.

The samples of soybean alcohol extracted cake, wheat, rice (polished), maize (entire kernel) and kaoliang (unpolished) flours employed in these experiments had the chemical composition as indicated in Table I.

Table I.

Kind of Flour	Moisture (%)	Crude Protein (%)	Ash (%)
Wheat No. A.	9.7	10.6	0.59
Wheat No. B.	10.1	13.5	0.47
Rice	11.8	6.9	0.83
Maize	12.5	7.0	1.20
Kaoliang	10.8	9.8	1.77
Soybean Cake	8.1	56.9	6.20

*Nutritive Value of the Wheat Flour and Soybean Cake
Flour Mixture.*

The young rats which were placed on a diet (No. F) consisting of wheat flour (85%) McCollum's salt mixture (5%), cod liver oil (3%), soybean oil (7%) and oryzanin (5 c.c. per 100 g. diet) failed to accomplish a good growth and all the female rate remained sterile. The animals, however, attained a normal growth when fed upon adiet (No. FACM5) composed of a 95 percent wheat flour and 5 percent soybean cake mixture, together with inorganic salts, cod liver oil, soybean oil and oryzanin similarly supplemented as in the previous diets. Female rats produced normal litters of young and suckled them successfully in most cases. The brood of the second generation placed on the diet the same as that on which their parents were raised made a fairly good growth.

Animals also secured an excellent growth, by two kinds of diets (No. FACM10 and No. FACM20) which contained the wheat flour 10 and 20 percent of which had been replaced with soybean cake flour and were similarly supplemented with other nutrients, their growth rates being similar to or even better than those of normal nutrition. Several broods of vigorous young were produced which were successfully suckled by mothers in most cases and grew normally on the same diets as those of their parents.

The growth curves of rats placed on these four diets are compared in Figure I.

*Nutritive Values of the Mixtures of Soybean Cake Flour
and other Cereal Flours.*

The results of our experiments demonstrated that the feeding of rats with rice, maize or kaoliang flour together with adequate amounts of inorganic salts and vitamins failed to induce in them a satisfactory growth, but when soybean cake flour was added to rice or maize flour at 10 per cent and to kaoliang flour at 20 per cent the mixtures were found to supply enough protein for the normal growth of white rats.

*Value of Protein of Soybean Cake to Supplement That of
Wheat Flour.*

Wheat flour "No. B" with a higher protein content was added with McCollum's salt mixture (5%), cod liver oil (3%), soybean oil (7%), oryzanin and starch to bring the protein content of diet up to a 10 per cent level. Another diet was prepared with the mixture of wheat flour and soybean cake flour (80:20) and similarly supplemented with other elements, it containing protein at 10 per cent, one half of which was furnished by wheat and another half by soybean cake.

Two groups of young rats were placed on these diets and their growth rates were compared with each other and also with those of rats on the diet in which the protein supplied by soybean cake was included at the same level. These comparisons showed that at a 10 per cent level of protein intake the mixed proteins derived from wheat flour (5%) and soybean cake (5%) were much greater in nutritive efficiency than the protein of soybean cake or of wheat flour supplied as the sole source.

These observations are in harmony with those of McCollum, Simmonds and Parsons⁽²⁾ who found that rats fed on a diet containing mixed proteins at 9 per cent (6 per cent from wheat and 3 per cent from soybean) grew much more rapidly than those on diets containing the same amount of protein derived from either wheat or soybean alone. We attributed these facts to the effects of soybean protein to supplementing the deficiencies in essential amino-acids of wheat protein, especially in lysine and arginine.

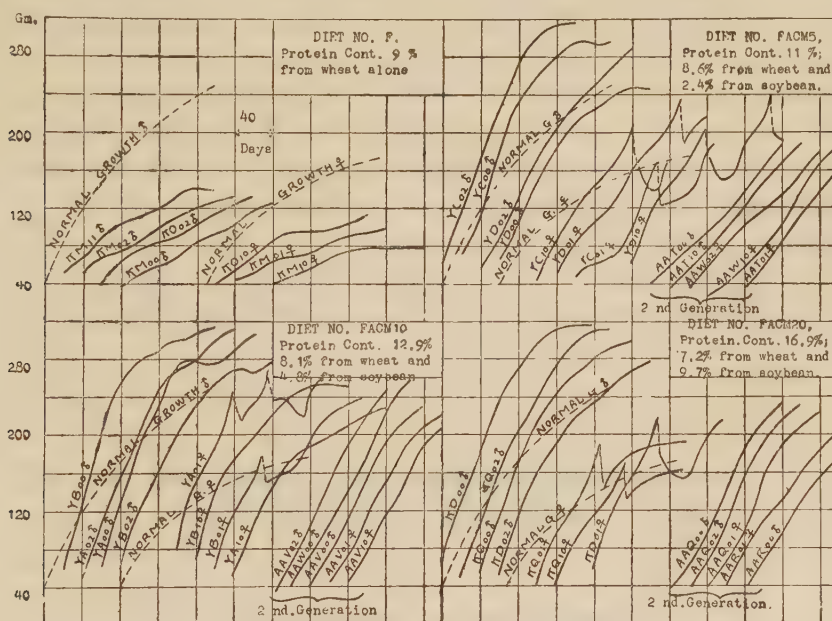


Fig. I. The growth curves of rats showing the differences in the nutritive value between wheat flour and the mixtures consisting of wheat flour and alcohol extracted cake soybean flour.

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The Influence of Ultra-violet Rays upon the Hatching Quality of Eggs.

By

Kozo SUZUKI and Tadashi HATANO.

(Imperial Zootechnical Experiment Station, Chiba, Japan)

(Received July 12, 1931)

Groups of eggs were exposed to the radiation of a "Silectra-Standard" quartz mercury-vapor lamp provided with a filter in periods varying from 15 minutes to 3 hours at a distance of 40 cm. from the lamp. In addition, groups of eggs unexposed to ultra-violet rays were incubated with those that had been exposed, and the following results were obtained.

1. The direct exposure of eggs to ultra-violet rays, whether irradiated just before placing them in the incubator or during the incubation processes, had no influence on the hatching quality of the eggs, whether white-shell or brown-shell eggs.

2. The exposure of the hen herself to ultra-violet rays will improve markedly the hatching quality of eggs, but eggs which are laid after the irradiation of the hen has been suspended are not influenced by the previous irradiation.

3. The wave length which can penetrate through the egg-shell measures about 3100 Å, but the egg-shell with the shell-membrane is opaque to radiation shorter than 3660 Å in wave length.

The Influence of Ultra-violet Rays upon the Production of the Egg.

By

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(Imperial Zootechnical Experiment Station, Chiba, Japan)

(Received July 12, 1931)

In order to ascertain the influence of the irradiation of ultra-violet rays on egg production in the winter season under the usual management of laying-hens, 36 pedigreed single comb white leghorn hens were selected and divided into two groups as uniformly in reference to laying history and breeding as could be done and each group was consisted of five 5-year-old

hens, three 3-year-old hens and ten pullets just begun laying.

These groups of hens were housed in two pens of the same construction and adjoining each other, and a male bird was placed in each of the two pens.

The following ration was given to both groups.

Mash	{	Wheat bran	150 parts
		Rice bran	150 "
		Barley bran	80 "
		Soybean cake	70 "
		Fish meal	50 "
Grain	{	Yellow corn	300 "
		Wheat	200 "

The ultra-violet light used was radiated by an "Acme Jesioneck" quartz mercury-vapor lamp. One group of hens was irradiated as a group 30 minutes daily at a distance of 1~2 meters for 120 days from November 1, 1928, to February 28, 1929; the other group was not irradiated.

We compared the number of eggs which were produced by both groups of hens during the above mentioned experimental period and further for 92 days after the suspension of the irradiation, from March 1 to May 31, 1929.

The following results were obtained:

1. The pullets, which had just begun laying, irradiated by ultra-violet rays produced more eggs than the non-irradiated ones, but the old hens, from 3 to 5 years old, were not consistently affected by the irradiation.

2. There was no noticeably bad effect upon the egg-laying after the suspension of the irradiation of ultra-violet rays.

3. The average daily food consumption per hen of both groups, irradiated and non-irradiated, was almost the same.

A New Method for the Estimation of Uric Acid in Poultry Excrement.

By

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(*Imperial Zootechnical Experiment Station, Chiba, Japan*)

(Received July 12, 1931)

1 g. of the dried and powdered urono-fecal mixture of poultry excrement is weighed into a beaker of 100 c.c. capacity and 20 c.c. of distilled water added. The contents of the beaker are now thoroughly mixed by stirring and brought gently to the boil on the sand bath. After boiling for about 1 minute, 5 c.c. of concentrated hydrochloric acid are added and the boiling

and stirring is continued further for a few seconds in order to convert the urates in the excrement into free uric acid and chlorides. The beaker is now set aside overnight in a cold place. The acid liquid is filtered off through a small filter paper and the residue on the filter paper is washed with distilled water which has been cooled with ice previously, until the washings contain no trace of hydrochloric acid and then treated with 90 per cent alcohol and ether successively in order to remove free fatty acids. Both the residue and the filter paper altogether are once more returned into the beaker and 20 c.c. of distilled water added. The contents of the beaker are again thoroughly mixed by stirring and brought to the boil on the sand bath. After boiling for some minutes, 5 c.c. of conc. ammonia liquid are stirred in and the boiling and stirring is continued further for a few seconds. The beaker is then allowed to stand for about 1 hour in a room temperature.

The free uric acid is converted into mono-ammonium urate by this treatment. The ammoniacal liquid in the beaker is now evaporated to dryness on the water bath and then the beaker is placed in the steam oven and heated at 100°C for some hours in order to remove the excess of ammonia completely. 20 c.c. of 5 per cent hydrochloric acid are now poured in the beaker and the contents of the beaker are again thoroughly mixed by stirring and boiled gently for about 1 minute on the sand bath. By this treatment, mono-ammonium urate is decomposed to the free uric acid and ammonium chloride.

The beaker is allowed to cool for some hours in order to crystallize out the uric acid completely. The acid liquid is now filtered off through the small filter paper and the residue on the filter is washed with cold distilled water which has been cooled with ice previously, till perfectly free from chloride.

The filtrate and washings are evaporated to about 10 c.c. on the water bath and then made up exactly 100 c.c. with absolute alcohol. 50 c.c. of the alcoholic solution are pipetted in the flask of a steam distilling apparatus and the excess of hydrochloric acid is neutralized with dilute alcoholic potash using phenolphthalein as an indicator and the 2 or 3 drops of the alcoholic potash are added to make the liquid faint alkaline.

The flask is connected with condenser and a rapid current of steam derived from distilled water which has been freed from CO₂ by prolonged boiling, is passed through the solution until a foam nearly filling the flask develops.

All ammonia in the solutions is distilled into a measured quantity of 1/5 or 1/10 normal solution of sulfuric acid. The excess of acid in the alcoholic contents of the receiver is determined by titrating with 1/10 normal solution of NaOH or KOH using alizarin (0.5 per cent alcoholic solution) as an indicator.

The amount of nitrogen of the distilled ammonia is estimated by the amount of alkali equivalent to the distilled ammonia.

In calculating the amount of uric acid, the amount of the estimated nitrogen may be multiplied by the factor 11.998 which is the ratio of a molecular weight of uric acid and an atomic weight of nitrogen.

The accuracy of this method for estimating uric acid in poultry excrement was confirmed by the following experiments.

Experiment I. A known amount of pure uric acid (Kahlbaum) was taken and estimated its quantity by our new method, and the following result was obtained.

Weight of pure uric acid taken	0.2989 g.
Amount of uric acid analysed	0.2985 g.
Error	0.13%

Experiment II. 0.5 g. of dried and powdered poultry urine which was collected separating from the feces by means of an artificial anus, was taken and its uric acid content was estimated. Then, a known amount of pure uric acid was added to 0.5 g. of the dried urine and total amount of uric acid was determined, and the quantity of uric acid originally added was calculated. The following result was obtained.

Weight of pure uric acid added	0.09963 g.
Amount of uric acid determined by analysis	0.09916 g.
Difference	0.00047 g.
Error	0.472%

Experiment III.

(A) 1.0 g. of dried and powdered poultry feces which was collected by means of a artificial anus and free from urinary constituents, was taken and treated to carry out the estimation of uric acid by our new method. We have obtained such result as the feces contained 0.336 per cent uric acid. Weighed amount of pure uric acid was submitted to the complete series of processes involved in the method and the following satisfactory result was obtained.

	(a)	(b)
Weight of pure uric acid added	0.09963 g.	0.1926 g.
Amount of uric acid analysed	0.09950 g.	0.20001 g.
Percentages of recovered amount	99.870%	100.376%
Error	0.130%	0.370%

(B). The feces which was used in Experiment III, (A) ought to contain no uric acid, but owing to the result of the analysis there existed 0.336 per cent uric acid in it. In order to ascertain definitely whether the incompleteness of the separation of urine and feces was due to obtain such result or the existence of certain volatile basic substances which were distilled by the steam distillation, interfered with the accuracy of the method, the bird whose

feces was used in Experiment III (A), was killed and the content of the small intestine was dried and then the uric acid estimation was carried out by our method. It resulted to show that the dried content of the small intestine contained 0.386 per cent uric acid.

It can be said by this experiment that some volatile basic substances in poultry feces are distilled by the steam distillation, but its amount is negligible.

Experiment IV. One bird (S. C. White Leghorn No. 3) was fed with soybean cake alone (high protein ration) and the other two (S. C. White Leghorn No. 1 and No. 2) were fed with kaoliang alone (low protein ration). Their excrements were collected separately, and the uric acid contents were determined by our method. The agreement between the duplicates, (a) and (b), in the trials is shown by the following figures.

	(a)	(b)
Uric acid content of the excrement of bird No. 1	8.168%	7.832%
" No. 2	7.008%	6.840%
" No. 3	26.27%	26.27%

Experiment V. Weighed amount of the pure uric acid was added to each excrement of the birds, which was used in Experiment IV, and total amount of uric acid was determined. Using the figures obtained in Experiment IV, the amount of uric acid originally added was calculated and satisfactory results were obtained, as shown by the following figures.

	Weight of uric acid added (g.)	Amount of uric acid determined by analysis (g.)	Difference (g.)	Error (%)
Experiment of No. 1	0.1993	0.19832	0.00098	0.161
No. 2	0.1993	0.19826	0.00104	0.572
No. 3 (a)	0.1993	0.19898	0.00032	0.161
(b)	0.1494	0.14991	0.00051	0.341

Studies on the Fermentation Products by Mould Fungi. Part IX.

By

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(Received June 2, 1931)

In parts IV and VIII of this series (Agr. Chem. Soc. Japan, **5**, 10, 1929; **6**, 106, 1930) it was shown that the 2-oxyethylfuran-5-carboxylic acid was

formed by *Asp. glaucus* under the particular cultural condition and the mechanism of the formation of this acid was cleared. In this paper, 15 kinds of *Aspergillus* have now examined whether to produce the 2-oxymethylfuran-5-carboxylic acid under this particular cultural condition and it comes to know that *clavatus*, *glaucus*, *niger*, *oryzae*, *wentii* produce this acid. Besides the formation of 2-oxymethylfuran-5-carboxylic acid, the interesting fact is the formation of a considerable amount of mannite by *Asp. clavatus*. As other fermentation products, ethyl alcohol, acetaldehyde, succinic, fumaric, oxalic, gluconic and acetic-acids are isolated and identified.

The fermentation products of every fungus are as follows.

- Asp. candidus*: Ethylalcohol, acetaldehyde, succinic acid, acetic acid.
- Asp. clavatus*: Ethylalcohol, acetaldehyde, succinic acid, 2-oxymethylfuran-5-carboxylic acid, mannite.
- Asp. fisheri*: Acetaldehyde, succinic acid, gluconic acid.
- Asp. flavus*: Ethylalcohol, acetaldehyde, fumaric acid.
- Asp. fumigatus*: Ethylalcohol, acetaldehyde, succinic acid, acetic acid
- Asp. glaucus*: 2-oxymethylfuran-5-carboxylic acid, gluconic acid.
- Asp. niger*: Ethylalcohol, acetaldehyde, succinic acid, 2-oxymethylfuran-5-carboxylic acid.
- Asp. oniki*: Acetic acid, fumaric acid.
- Asp. oryzae* T. N.: Ethylalcohol, acetaldehyde, 2-oxymethylfuran-5-carboxylic acid.
- Asp. ostianus*: Ethylalcohol, acetaldehyde, succinic acid, gluconic acid.
- Asp. pulverentus*: Ethylalcohol, acetaldehyde, oxalic acid, gluconic acid.
- Asp. sydowi*: Ethylalcohol, acetaldehyde.
- Asp. terreus*: Ethylalcohol, acetaldehyde, succinic acid.
- Asp. versicolor*: Ethylalcohol, acetaldehyde, acetic acid.
- Asp. wentii*: 2-oxymethylfuran-5-carboxylic acid, fumaric acid.

Über die physiologischen Wirkung der Phytosterin-ester.

Von

Zirō NIKUNI.

(Eingegangen Juni 8, 1931)

Zur Erklärung der physiologischen Wirkung der Phytosterin-ester machte der Autor das vergleichenden Tierexperiment zwischen den Cholesterin- und Phytosterin-estern (Acetat und Palmitat).

Die Resultate sind folgend:

1. Zur Ernährung der weissen Ratten hat Phytosterin-ester sowie Cholesterin-ester die ansehnlichen Schädigungskraft nicht. Und zwischen den beiden Sterinen ist kein klarer Unterschied. Aber im allgemeinen steht der Acetat der beiden Sterine dem Palmitat an der Ernährung der Tiere nach.

2. Die Sterin-ester, die von Munde gegeben sind, ausscheiden etwa die

Hälfte der Menge im Kot als die gegebenden Formen. Die Ausscheidungs-
menge der Phytosterin-ester ist gering wenig als die der Cholesterin-ester.
Im allgemeinen ist die Ausscheidungs-
menge der Palmitat grösser als die der
Acetat.

3. Der Sterin-gehalt der experimentierten Tiere ist grösser bei der mit
Cholesterin-ester gefütterten Tieren. Der Sterin-gehalt, der mit Phytosterin-
ester gefütterten Tiere ist doch grösser als der der kontrollierten Tiere.

4. Das Körpersterin der mit Phytosterin-ester gefütterten Tiere enthält
die kleine Menge des Phytosterins.

Der Schluss.

Das Phytosterin, das mit dem Nahrungsmittel gegeben ist, ist ein Teil
absorbiert und setzt sich im Tierkörper wie das Cholesterin.

Und die absorbierenden und setzenden Grade der beiden Sterine sind zu
ihren Esterformen abhängig.

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